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Original Article

CSE1L silencing impairs tumor progression via MET/STAT3/PD-L1 signaling in lung cancer

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Abstract: CSE1L is involved in the cancer progression of several types of cancer. Its expression status, potential oncogenic role and underlying mechanism in lung cancer, however, are unclear. Here, we investigated CSE1L expression in primary lung adenocarcinoma based on multiple datasets and then investigated its oncologic role in lung cancer. We also examined the potential molecular mechanisms of CSE1L in cancer progression. *CSE1L* levels were increased in cancer as compared to normal lung tissues. *CSE1L* expression was higher in poorly-differentiated late stage and lymph node positive metastatic tumors. Higher *CSE1L* level was correlated with worse patient outcome. Knockdown of CSE1L using siRNAs impaired cell proliferation, invasion, migration and induced cell apoptosis. Mechanistically, MET, STAT3 and PD-L1 proteins were decreased upon CSE1L silencing. These results suggest that CSE1L may affect tumor progression through MET/STAT3/PD-L1 signaling. CSE1L may have potential as a biomarker and therapeutic target for lung cancer.

Keywords: Lung cancer, CSE1L, MET, STAT3, PD-L1

Introduction

Lung cancer new cases and deaths are increasing. There are 1.76 million deaths from lung cancer worldwide in 2018 [1, 2]. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancer and adenocarcinoma (AD) represents the predominant histological type, accounting for over half of all NSCLC. Despite intensive research and several therapeutic advances in lung cancer, the overall 5-year patient survival is only 20% [2]. Consequently, it is critical to discover more successful treatments that might either delay progression or improve patient survival in lung cancer [3, 4].

CSE1L (Chromosome Segregation 1-Like) is located on 20q13.13, and encodes a 971-amino-acid protein [5]. It associates with microtubules and mitotic spindles, and is also considered to have a role in tumor progression [6]. The CSE1L protein includes a nuclear localization signal allowing for transport into the nucleus via the importin-alpha/beta heterodimer. In addition, the encoded protein plays roles in cell proliferation and apoptosis [7, 8]. Studies suggest that CSE1L is a potential oncogene [9-11] that is highly expressed in several tumors including colorectal, ovarian and gastric and has a critical role in cancer development by regulating MITF, GPNMB, AKT or MEK signaling [11-19]. CSE1L can regulate the MAPK pathway via interaction with p65 in lung cancer [20], but the expression status, oncogenic roles and underlying molecular mechanisms of CSE1L still remains unclear.

Here, we systematically evaluated *CSE1L* expression using RNA-seq and microarray data from several large cohorts of primary lung cancer samples. *CSE1L* was overexpressed in lung cancer and the higher expression was correlated with unfavorable patient survival. The differential expression of *CSE1L* was then validated

in an independent cohort of 101 ADs using qRT-PCR. Functional examination of CSE1L revealed that upon CSE1L knockdown using siRNA, cell proliferation, invasion and migration were reduced. Mechanistically, MET, STAT3 and PD-L1 proteins were decreased upon CSE1L silencing. All these results suggest that CSE1L-regulated cell proliferation and anti-apoptosis effects may be via MET/STAT3 signaling in lung cancer cells.

Materials and methods

Lung cancer tissues and cell lines

The lung cancer tissue samples utilized for both RNA-sequencing and qRT-PCR validation were as described in our previous studies [21, 22]. These samples were obtained from patients who had undergone curative tumor surgery during the period from 1995 to 2016 at the University of Michigan (UM) Health System and did not receive preoperative radiation or chemotherapy. The median follow-up time of patients was 9 years. All patients provided informed consent and this project received approval from the UM Institutional Review Board and Ethics Committee. All lung cancer cell lines were purchased from the ATCC (American Type Culture Collection). Cells were cultured at 37°C in a 5% CO_2 cell culture incubator. Cells were genotyped for identity validation at the UM sequencing core and were tested routinely for Mycoplasma contamination.

RNA extraction and qRT-PCR

Total RNA extraction was performed from cell lines and tissue samples utilizing the miRNeasy Mini kit (Qiagen), and cDNA was obtained using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). The qRT-PCR reaction was performed using Power SYBR Green master Mix (Life Technology Inc.) with ABI Step One Real-Time PCR System (Applied Biosystems). The reactions for each sample had a final volume of 15 µL and contained approximately 20 ng of cDNA. The oligonucleotide primers for CSE1L included: forward, 5'-CAAGTGCTGCAGCTGACAAA-3', and reverse, 5'-CTTGTAGTGCTAGTGCCCCA-3'. The loading control genes were GAPDH and ACTB. Relative mRNA levels were assessed using the 2 delta Ct method [23].

Immunoblot analysis

For preparing protein samples, tissues or cells were lysed in RIPA buffer accompanied with protease inhibitors cocktail (Sigma) and denatured by boiling. The proteins were electrophoresed by SDS-PAGE and transferred onto the nitrocellulose membranes (Millipore Corporation). The transferred membranes were blocked in 5% non-fat milk (Thermo Fisher Scientific), following the incubation of the primary antibodies and the secondary antibodies, respectively. After removal of excess antibodies with repeated washes, the ECL developed membranes were exposed to the ChemiDoc MP Imaging System (BIO-RAD). Tissue microarray from formalin-fixed, paraffin-embedded tumors including 92 ADs was examined using immunohistochemistry with a CSE1L antibody (ab54674, Abcam) and performed as described previously [24-26].

siRNA transfection

The detailed methods for siRNA transfection are as described in our previous studies [22, 27]. For CSE1L knockdown, lung cancer cells were treated with the transfection reagents Lipofectamine RNAi MAX reagent (Invitrogen, USA) and 10 nM ON-TARGET plus SMART pool Human CSE1L siRNAs.

Cell proliferation, invasion and migration

The WST-1 assay (Roche) used for cell proliferation was performed according to the manufacturers' instructions. A wavelength of 450 nm was used and the analysis of cell viability was performed by normalization to the non-target siRNA group. The migration and invasion abilities of tumor cells were evaluated using Boyden chambers (8-µm, BD Biosciences). The invasion ability of cells was measured with the chambers coated with 100 µl Matrigel (BD Company) for 4 h and migration ability was measured in chambers without Matrigel. After incubation in the above culture conditions for 24 h, the invading cells in the bottom chamber were fixed with 20% methanol and stained with crystal violet. The plates were photographed using an inverted microscope (measuring 5 fields per membrane). The stained cells were then counted for subsequent statistical analysis. Each experiment was performed in triplicate.

Collection of published RNA-seq and microarray data

Four published microarray datasets containing 759 NSCLC and 65 adjacent normal lung were collected. These consist of the Shedden et al., dataset (442 AD) [28], Su et al., dataset (27 AD and 27 normal lung tissues) [29], Tomida et al., dataset (117 AD) [30] and Hou et al., dataset (65 normal lung tissue samples and 91 NSCLC tissues including 45 AD and 19 LC and 27 SCC) [31]. The original CEL files were analyzed using the RMA (Robust Multi-array Average) method [32]. RNA-seq datasets from lung cancers included the datasets from UM (67 AD, 36 SCC, 10 LC and 6 normal lung tissues) [33], TCGA (309 AD, 212 SCC and 73 normal lung tissues) [34] and Seo et al., (85 AD and 77 normal lung tissues) [35]. Expression levels of mRNAs were defined as RPKM [36].

Statistical analysis

Data were analyzed with R software, excel or GraphPad Prism 6. Kaplan-Meier survival curves were used for survival analysis. With the median of *CSE1L* mRNA as a cutoff, the survival differences were assessed using the log-rank test. Pearson's correlation coefficient was used for the correlation analysis between *CSE1L* mRNA and other genes. Student's t-test was used for cell proliferation, migration and invasion assays. The Gene Ontology and pathways in DAVID website [37] were applied to establish potential biological processes or pathways associated with CSE1L correlated genes.

Results

CSE1L mRNA is highly expressed in NSCLC

To evaluate the *CSE1L* mRNA expression status in NSCLC, we explored multiple microarray and RNA-seq datasets of NSCLC [28, 29, 31, 33]. *CSE1L* mRNA expression was higher in lung cancer (including AD, LC and SCC, relative to normal) in all datasets (**Figure 1A-E**). This was confirmed by qRT-PCR validation set including 101 ADs and 20 normal lung tissues (P<0.001) (**Figure 1F**). In the Shedden dataset [28], *CSE1L* mRNA was higher in poorly differentiated (vs. well, P=0.01) as well as in late-stage tumors (stage 3 vs. stage 1, P<0.001) (**Figure 1G**, **1H**). In lymph node positive and thus metastatic tumors, *CSE1L* mRNA was increased in N1-2 vs. N0 tumors (P<0.001) (**Figure 1I**). These results indicated that *CSE1L* is both highly expressed and may have potential as a biomarker for lung cancer. We also examined *CSE1L* mRNA expression in other cancer types in TCGA data from the GEPIA website, and found that *CSE1L* mRNA is increased in all different types of cancers except LAML (Acute Myeloid Leukemia) (Figures S1, S2).

Overexpression of CSE1L mRNA is unfavorable for survival in AD patients

To test whether *CSE1L* expression is related to patient survival, we evaluated two independent AD microarray datasets where survival information was provided including the Shedden et al. [28] and Tomida et al. datasets [30]. We found that higher *CSE1L* mRNA expression was associated with a poorer patient survival in both data sets using Kaplan-Meier survival curve analysis (log-rank test, P<0.0001 and P=0.02, respectively) (**Figure 2A**, **2B**). Similar results were observed in an RNA-seq cohort from UM (P=0.006) (**Figure 2C**). We further measured *CSE1L* mRNA expression in an expanded UM 101 AD cohort by qRT-PCR analysis. The result indicated that higher *CSE1L* mRNA expression was significantly correlated with unfavorable survival in AD (**Figure 2D**). We also evaluated *CSE1L* mRNA expression and survival in TCGA lung adenocarcinoma RNA-seq data from the GEPIA website. Higher levels of *CSE1L* mRNA is correlated with poor survival in patients with AD (Log-rank test, P=0.03) (Figure S3). Taken together, these results indicated that *CSE1L* has potential as a prognostic marker for AD.

CSE1L protein expression is increased in AD tissues

To examine if CSE1L protein is similarly overexpressed in lung cancer, we utilized immunohistochemistry (IHC) using a tumor tissue microarray (TMA). Tumor-specific CSE1L protein expression was scored based upon intensity of CSE1L positivity as 0, 1 or 2. Tumors were then grouped into score 0 (**Figure 3A**), score 1 (**Figure 3B**) and score 2 (**Figure 3C**, **3D**). More than half of tumors demonstrated CSE1L positive staining (**Figure 3E**), with the majority showing nuclear localization (**Figure 3E**, **3F**). Further, Western blot analysis confirmed higher CSE1L protein expression in tumor tissues relative to

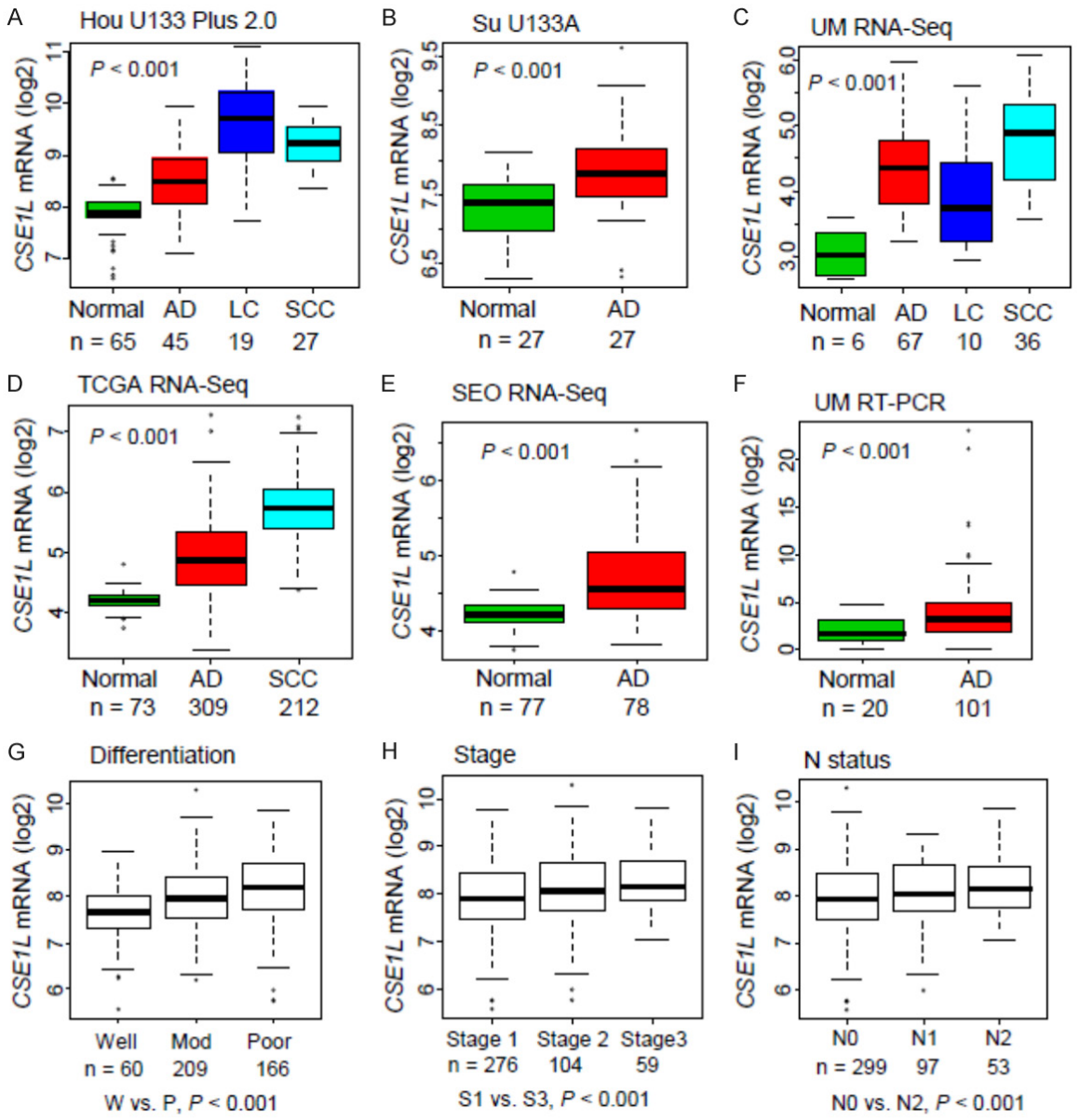

Figure 1. *CSE1L* mRNA was overexpressed in lung cancer. A-E. *CSE1L* mRNA expression is higher in lung cancer as compared to normal lung tissues using several lung cancer datasets utilizing both microarray and RNA-seq. F. *CSE1L* mRNA expression was examined with an independent cohort of lung cancer using qRT-PCR. G and H. *CSE1L* mRNA is higher in poor vs. well differentiated tumors (P=0.01), and in late-stage tumors (stage 3 vs. stage 1, P<0.001, Shedden et al., dataset). I. *CSE1L* mRNA was increased in tumors demonstrating positive lymph node metastasis N1-2 vs. N0 (P<0.001, Shedden et al., dataset). AD, lung adenocarcinoma; SCC, squamous cell lung cancer; LC, large cell lung cancer.

normal lung tissues taken from the same patients (**Figure 3H-J**). This data indicates that both CSE1L mRNA and protein are higher in lung tumors.

CSE1L knockdown inhibits cell proliferation, migration and invasion in lung cancer

To examine the potential oncogenic role of CSE1L in lung cancer, we performed cell proliferation, invasion and migration assays following CSE1L knockdown in the lung cancer cell lines H23, HCC78 and H1299. The CSE1L siRNAs (SmartPool) knockdown efficiency was 80%-90%, which was confirmed using both qRT-PCR (**Figure 4A**) and Western blot. We found a substantial decrease in cell proliferation after the treatment of CSE1L siRNAs in all three lung cancer cell lines (**Figure 4B**). Transwell assays were utilized for evaluating tumor

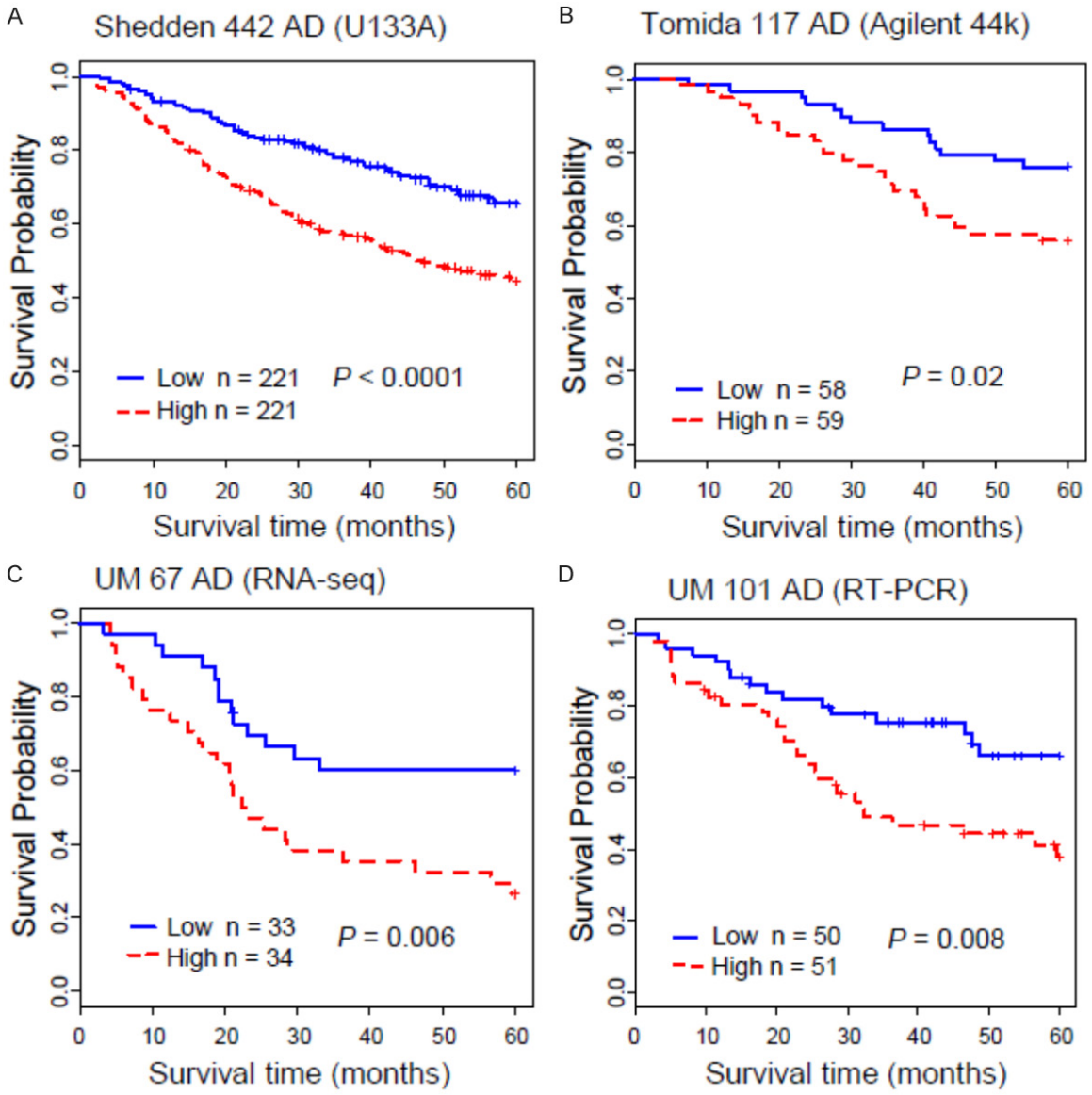

Figure 2. High levels of *CSE1L* mRNA are correlated with unfavorable survival in AD. A-C. Kaplan-Meier survival curve showing that patients with higher *CSE1L* mRNA level have a worse survival in lung cancer using the microarray datasets of Shedden et al., (log-rank test, *P*<0.0001) and Tomida et al., (*P*=0.02), as well as using the UM RNA-seq dataset (*P*=0.006). D. Association with patient survival was validated with qRT-PCR in an independent cohort of AD (*P*=0.008).

cell invasion and migration upon CSE1L knockdown in H23, H1299 and HCC78 cells. Cell invasion and migration were reduced upon treatment with CSE1L siRNAs (*P*<0.001) (**Figure 4C-F**). These data indicate that CSE1L may have an oncogenic role in lung cancer.

CSE1L correlated genes participate in cell cycle regulation

To define the potential pathway involved with CSE1L, we performed Pearson correlation assessment between *CSE1L* and all coding genes using RNA-seq data from three human primary NSCLC cohorts including a total of 461 ADs (Seo et al., 85 ADs, UM 67 ADs and TCGA 309 ADs) [22, 33]. There were 979 coding genes demonstrating a positive correlation with *CSE1L* (r>0.4, n=461, *P*<0.001). Based on DAVID pathway and gene annotation assessment using the *CSE1L* positive correlated genes, cell cycle regulation was the top pathway (**Figure 5A**). For example, cell cycle associated genes, *CHEK1* and *CDK1* were significant-

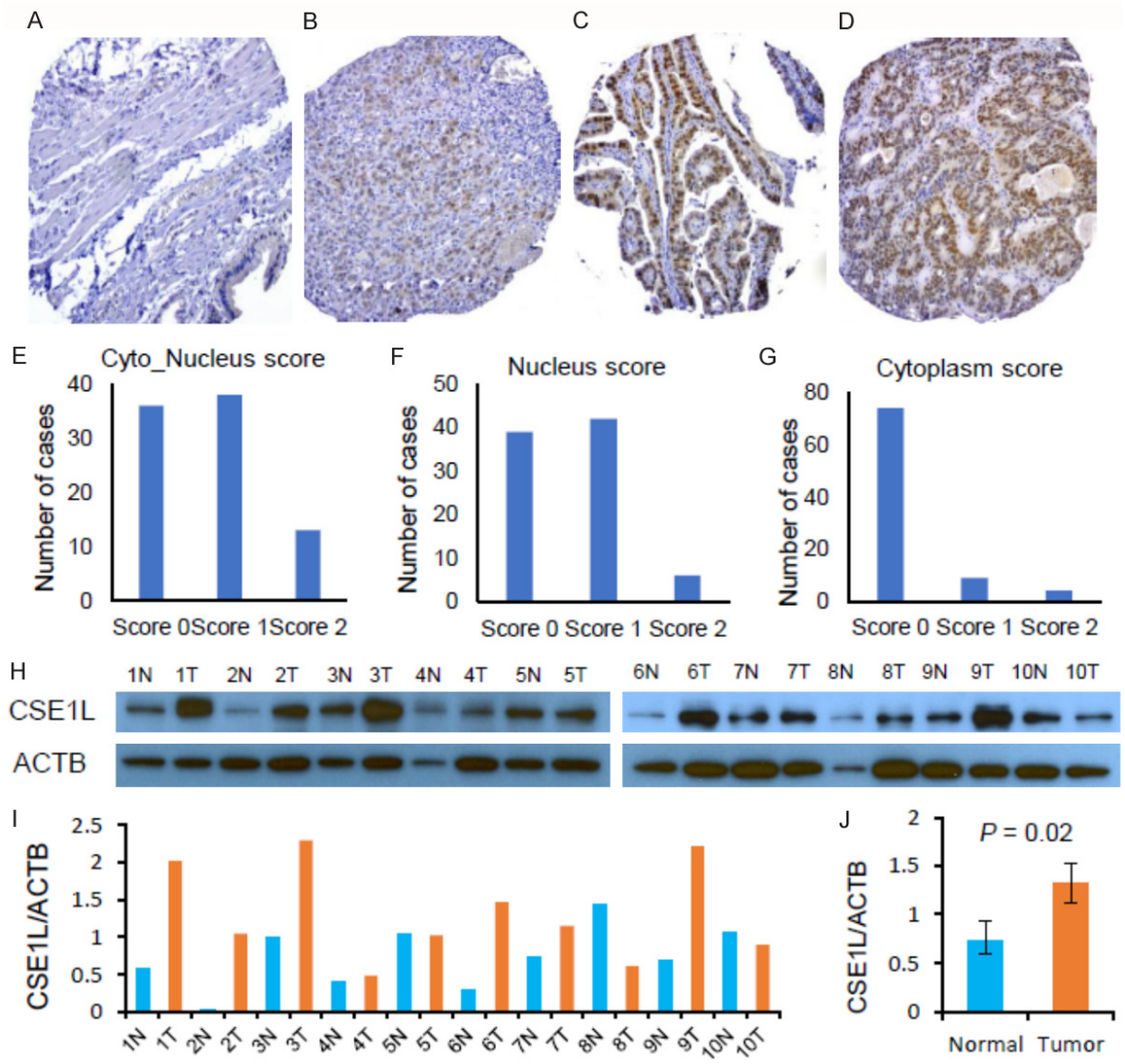

Figure 3. CSE1L protein expression in lung cancer. (A-D) CSE1L protein IHC (immunohistochemistry) staining status examined using a lung AD TMA (tissues array). Representative images scored as 0 (A), score 1 (B) and score 2 (C and D). (E-G) CSE1L protein is expressed both in the nucleus and in the cytoplasm but is predominantly found in the nucleus. (H) Western blot showing higher CSE1L protein expression in tumors as compared to paired normal lung tissues. (I and J) Quantitative analysis of the intensity of (H) using ImageJ software for individual samples and the average value of tumors and normal lung tissues.

ly correlated with *CSE1L* expression in NSCLC (**Figure 5B**, **5C**). Other pathways included DNA replication and spliceosome, RNA transport, DNA repair and metabolism. These results suggest that CSE1L participates in several pathways in lung tumors.

Silencing of CSE1L decreases MET, STAT3 and PD-L1 protein expression and induces apoptosis

To understand the underlying oncogenic mechanisms of CSE1L in lung cancer, we performed CSE1L knockdown with siRNA treatment in lung cancer cells followed by Western blot analysis. We observed that the important lung cancer oncogenic protein MET was significantly reduced upon CSE1L silencing in H1299 and HCC78 cells. STAT3 was also down regulated in HCC78 cells (**Figure 6A**). Further, reduced MET and STAT3 proteins were further confirmed in H1650, A549 and PC-9 lung cancer cells (**Figure 6B**). We observed that the apoptosis related marker protein c-PARP was induced upon CSE1L silencing, suggesting CSE1L is involved in cell apoptosis (**Figure 6A**, **6B**).

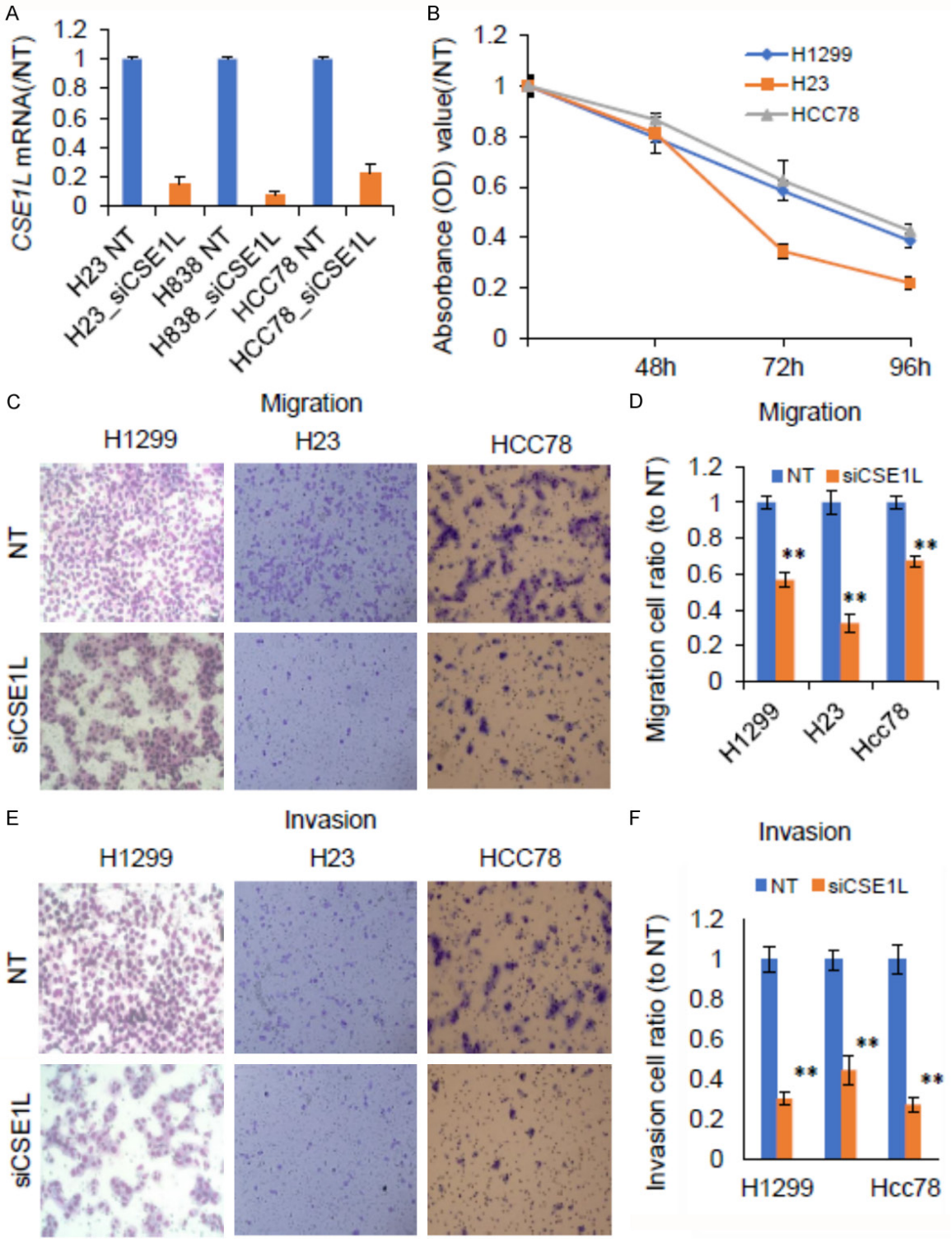

Figure 4. CSE1L silencing impairs cells proliferation, migration and invasion in lung cancer cells. A. *CSE1L* mRNA expression was significantly decreased by 80%-90% upon CSE1L siRNAs treatment for 48 hrs as measured by RT-PCR in H23, H838 and HCC78 cells. B. Cell proliferation was impaired after knockdown of CSE1L using siRNAs at 72 or 96 hrs and measured by WST-1 assay in H23, H1299 and HCC78 cells. C-F. Cell invasion and migration were inhibited by CSE1L silencing in all three lung cancer cells.

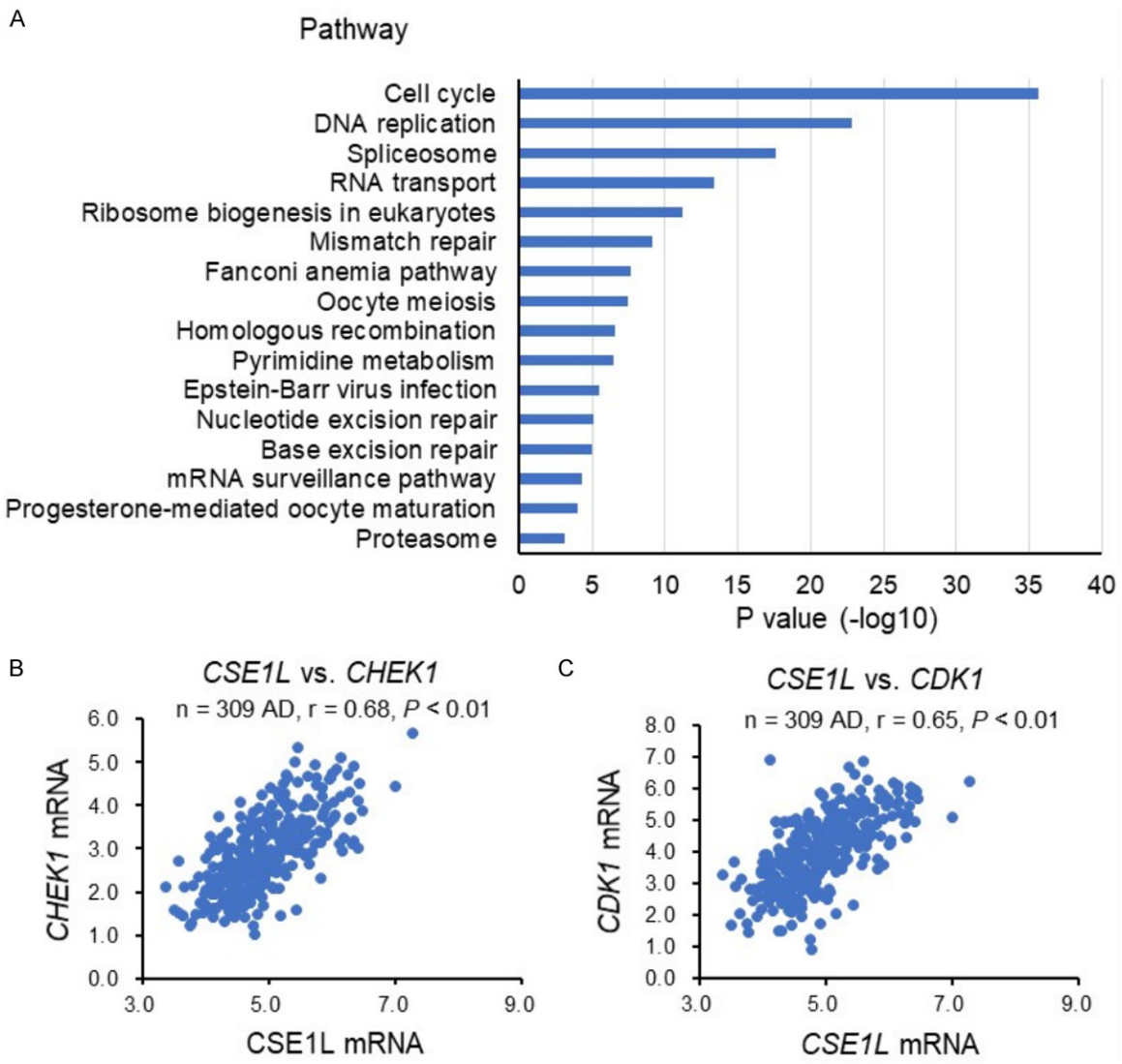

Figure 5. *CSE1L* correlated genes are involved in cell cycle regulation. A. DAVID web-based analysis of pathways associated with *CSE1L* positive genes. Cell cycle was the most significantly associated pathway among the *CSE1L* related genes. B and C. Cell cycle related genes, *CHEK1* or *CDK1*, were significantly correlated with *CSE1L* mRNA expression in AD.

As PD-L1 is reported to be regulated by MET signaling [38, 39], we tested whether PD-L1 protein was also affected by CSE1L. We found that PD-L1 protein levels were significantly decreased after CSE1L silencing in 5 out of 6 tested lung cancer cells, while H23 cells showed no PD-L1 expression (**Figure 6C**). Further, PD-L1 protein expression was also decreased upon MET knockdown (**Figure 6D**), indicating that CSE1L may regulate PD-L1 via MET in these cells. The mRNAs of *MET*, *STAT3* and *PD-L1* were not changed upon CSE1L silencing in lung cancer cell lines (**Figure 6E-G**) and we observed no correlation between *CSE1L* mRNA and *PD-L1* (*CD274*) mRNA in NSCLC (**Figure 6H**), suggesting that CSE1L regulation of MET, STAT3 and PD-L1 was at the post-transcriptional level. Further future detailed mechanistic studies are warranted. Taken together, MET and STAT3 are important proteins for cell proliferation, cell survival as well as being therapeutic targets in lung cancer [40-42]. STAT3 and PD-L1 have been reported to be downstream of MET signaling [38-40]. We have tested additional cancer related proteins such as AKT, ERK1/2, S6K, YBX1, SLUG, p62, AMPKa, LC3B and N-cadherin by Western blot but didn't find a consistent changing (Figure S4). Taken together, our

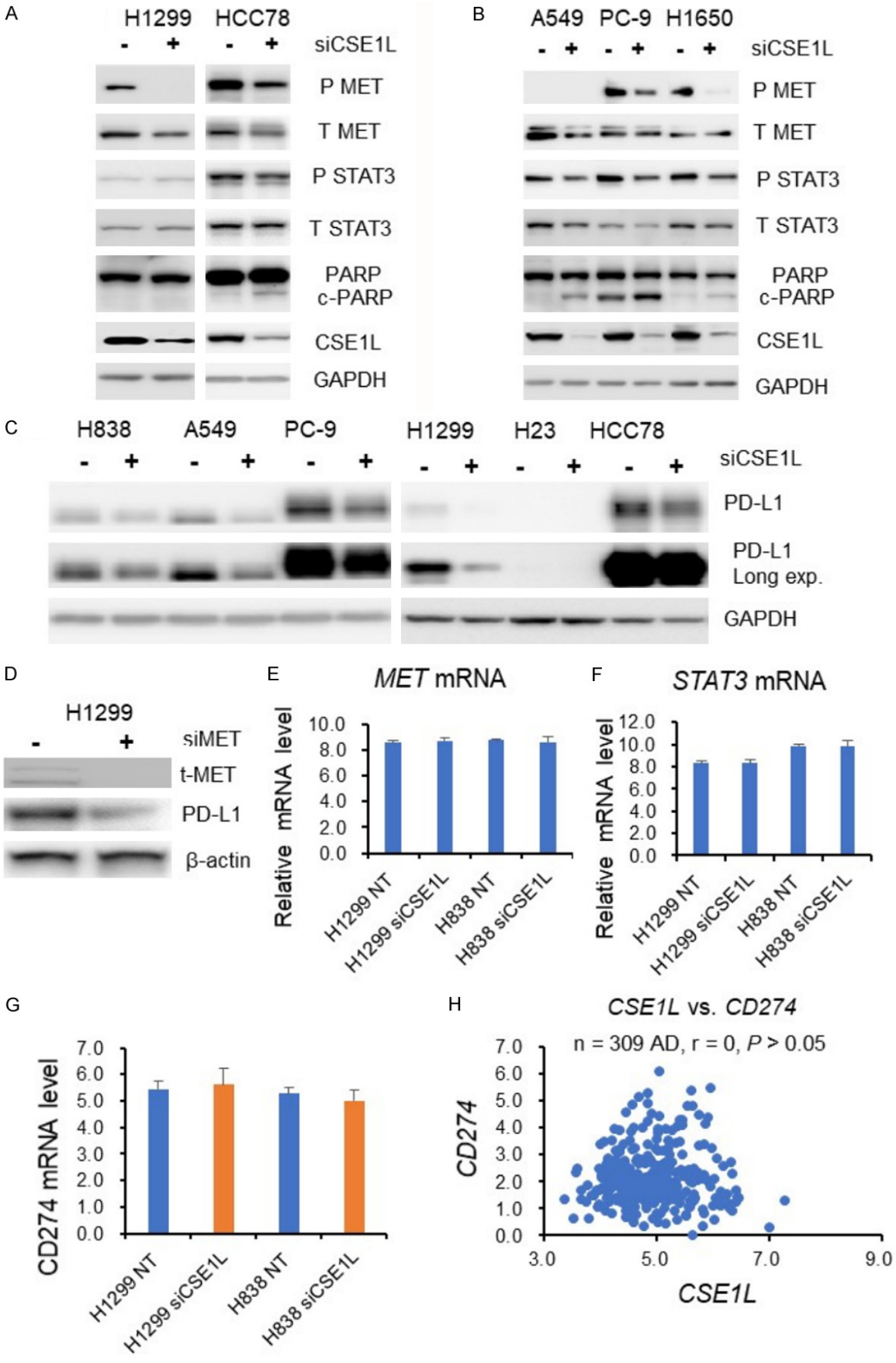
A
H1299
HCC78
siCSE1L
P MET
T MET
P STAT3
T STAT3
PARP
c-PARP
CSE1L
GAPDH
B
A549
PC-9
H1650
C
H838
H23
PD-L1
PD-L1
Long exp.
D
siMET
t-MET
β-actin
E
MET mRNA
Relative mRNA level
H1299 NT
H1299 siCSE1L
H838 NT
H838 siCSE1L
F
STAT3 mRNA
G
CD274 mRNA level
H
CSE1L vs. CD274
n = 309 AD, r = 0, P > 0.05
CD274
CSE1L

Figure 6. MET, STAT3 and PD-L1 proteins were decreased after CSE1L silencing. A-C. MET, STAT3 and PD-L1 proteins were decreased after CSE1L siRNAs treatment at 72 hrs in the lung cancer cell lines H1299, HCC78, A549, H838, PC-9 and H1650. GAPDH was used as loading control. The apoptotic marker cleaved-PARP (c-PARP) was induced following CSE1L silencing. D. PD-L1 protein was decreased upon MET silencing. E-G. *MET*, *STAT3* and *CD274* (*PD-L1*) mRNAs were not changed upon CSE1L knockdown with siRNAs in H1299 and H838 cells. H. *CD274* mRNA expression was not correlated with *CSE1L* mRNA in lung AD (n=309, r=0, *P*>0.05).

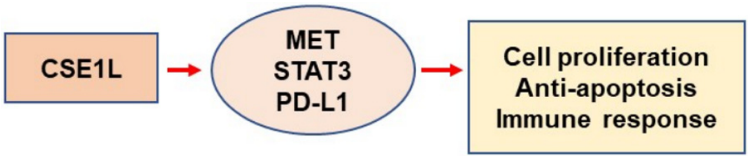

Figure 7. Schematic model showing that CSE1L regulates tumor progression via MET/STAT3/PD-L1 signaling pathway.

results indicate that CSE1L mediates lung cancer progression may be via MET/STAT3/PD-L1 signaling.

Discussion

CSE1L is overexpressed in several types of cancers including colorectal, ovarian, gastric, melanoma, larynx, bladder, osteosarcoma, hepatocellular and breast cancers [9, 11-14, 17, 43-52], as well as being reported to be associated with patient survival in bladder and osteosarcoma cancers [50, 51]. Cytoplasmic CSE1L expression is correlated with distant or lymph node metastasis in gastric, melanoma and breast cancer [13, 17, 53-55]. Recently, Lin et al. described that CSE1L was overexpressed and correlated with poor patient survival in NSCLC but utilized limited samples [20]. In this study, we comprehensively analyzed *CSE1L* expression in four microarray and three RNA-seq datasets, revealing that *CSE1L* levels are substantially higher in NSCLC. This was further confirmed using in a cohort of 101 ADs using qRT-PCR. Overexpression of *CSE1L* mRNA was also significantly correlated to poor patient prognosis in several public datasets and confirmed in our independent RT-PCR validation assessment. The CSE1L protein was also overexpressed in lung cancer tissues as measured by Western blot. Using IHC, CSE1L protein was highly expressed in both the nucleus and cytoplasm but tended to show strong nuclear staining in lung tumor tissue.

CSE1L was reported to be an oncogene and can inhibit apoptosis and promote cell proliferation in several cancer types [11, 17, 56]. Knockdown of CSE1L attenuates seminoma cells proliferation by arresting them in the G0/G1 and G2/M phase [57]. In our study, we found that CSE1L silencing impaired cell proliferation, invasion and migration of lung cancer cells, suggesting CSE1L plays an essential oncogenic role in cancer progression.

Potential underling mechanisms of CSE1L action has been reported in several kinds of cancer [6]. CSE1L inhibition can activate PI3K/AKT/mTOR and MEK/ERK by decreasing MITF and GPNMB in gastric cancer [17]. CSE1L suppression reduced drug resistance in melanoma cells by inhibiting RAS/ERK and cAMP/PKA pathways [12]. CSE1L can interact with p65 and regulate MAPK signaling in lung cancer [20]. We found that STAT3 and MET protein expression were decreased and the apoptotic marker cleaved-PARP was induced upon CSE1L silencing, suggesting CSE1L-regulation of cell proliferation and anti-apoptosis may be though the MET-STAT3 pathway. Further investigation of the detailed mechanism both *in vitro* and *in vivo* are warranted and planned in our future studies.

PD-L1 is an important immune checkpoint protein and immunotherapeutic target [58, 59]. Ahn and Saigi et al. stated that the expression of PD-L1 was decreased upon MET knockdown with siRNA/shRNA or MET inhibitors indicating PD-L1 was involved in MET signaling in lung cancer [38, 39]. We also found that MET and PD-L1 proteins were reduced upon CSE1L silencing in multiple cells containing different genetic alterations. PD-L1 was also decreased upon MET silencing, supporting that CSE1L may be involved in immune response through the regulation of MET/PD-L1 signaling pathway. Further detailed mechanistic analyses are clearly warranted.

Taken together, in the present study, we have systematically investigated CSE1L expression, its oncogenic roles and potential molecular mechanism in lung cancer, supporting its role as potential driver of lung cancer pathogenesis (**Figure 7**). CSE1L may have potential as a diagnostic/prognostic biomarker, and further stud-

ies may define its potential as a therapeutic target for lung cancers.

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Disclosure of conflict interest

None.

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References

[1] Mattiuzzi C and Lippi G. Current cancer epidemiology. J Epidemiol Glob Health 2019; 9: 217-222.

[2] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021; 71: 7-33.

[3] Kim N, Kim HK, Lee K, Hong Y, Cho JH, Choi JW, Lee JI, Suh YL, Ku BM, Eum HH, Choi S, Choi YL, Joung JG, Park WY, Jung HA, Sun JM, Lee SH, Ahn JS, Park K, Ahn MJ and Lee HO. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. Nat Commun 2020; 11: 2285.

[4] Goveia J, Rohlenova K, Taverna F, Treps L, Conradi LC, Pircher A, Geldhof V, de Rooij L, Kalucka J, Sokol L, Garcia-Caballero M, Zheng Y, Qian J, Teuwen LA, Khan S, Boeckx B, Wauters E, Decaluwe H, De Leyn P, Vansteenkiste J, Weynand B, Sagaert X, Verbeken E, Wolthuis A, Topal B, Everaerts W, Bohnenberger H, Emmert A, Panovska D, De Smet F, Staal FJT, McLaughlin RJ, Impens F, Lagani V, Vinckier S, Mazzone M, Schoonjans L, Dewerchin M, Eelen G, Karakach TK, Yang H, Wang J, Bolund L, Lin L, Thienpont B, Li X, Lambrechts D, Luo Y and Carmeliet P. An integrated gene expression landscape profiling approach to identify lung tumor endothelial cell heterogeneity and angiogenic candidates. Cancer Cell 2020; 37: 21-36, e13.

[5] Brinkmann U, Brinkmann E, Gallo M and Pastan I. Cloning and characterization of a cellular apoptosis susceptibility gene, the human homologue to the yeast chromosome segregation gene CSE1. Proc Natl Acad Sci U S A 1995; 92: 10427-10431.

[6] Jiang MC. CAS (CSE1L) signaling pathway in tumor progression and its potential as a biomarker and target for targeted therapy. Tumour Biol 2016; 37: 13077-13090.

[7] Behrens P, Brinkmann U and Wellmann A. CSE1L/CAS: its role in proliferation and apoptosis. Apoptosis 2003; 8: 39-44.

[8] Bera TK, Bera J, Brinkmann U, Tessarollo L and Pastan I. Cse1l is essential for early embryonic growth and development. Mol Cell Biol 2001; 21: 7020-7024.

[9] Lorenzato A, Biolatti M, Delogu G, Capobianco G, Farace C, Dessole S, Cossu A, Tanda F, Madeddu R, Olivero M and Di Renzo MF. AKT activation drives the nuclear localization of CSE1L and a pro-oncogenic transcriptional activation in ovarian cancer cells. Exp Cell Res 2013; 319: 2627-2636.

[10] Tong CY, Hui AB, Yin XL, Pang JC, Zhu XL, Poon WS and Ng HK. Detection of oncogene amplifications in medulloblastomas by comparative genomic hybridization and array-based comparative genomic hybridization. J Neurosurg 2004; 100: 187-193.

[11] Liang C, Wang X, Zhang Z, Xiao F, Feng H, Ma Q, Huang J, Qiang G, Zhong D and Liu D. ACOT11 promotes cell proliferation, migration and invasion in lung adenocarcinoma. Transl Lung Cancer Res 2020; 9: 1885-1903.

[12] Lee WR, Shen SC, Wu PR, Chou CL, Shih YH, Yeh CM, Yeh KT and Jiang MC. CSE1L Links cAMP/PKA and Ras/ERK pathways and regulates the expressions and phosphorylations of ERK1/2, CREB, and MITF in melanoma cells. Mol Carcinog 2016; 55: 1542-1552.

[13] Yuksel UM, Turker I, Dilek G, Dogan L, Gulcelik MA and Oksuzoglu B. Does CSE1L overexpression affect distant metastasis development in breast cancer? Oncol Res Treat 2015; 38: 431-434.

[14] Zhu JH, Hong DF, Song YM, Sun LF, Wang ZF and Wang JW. Suppression of cellular apoptosis susceptibility (CSE1L) inhibits proliferation and induces apoptosis in colorectal cancer cells. Asian Pac J Cancer Prev 2013; 14: 1017-1021.

[15] Li KK, Yang L, Pang JC, Chan AK, Zhou L, Mao Y, Wang Y, Lau KM, Poon WS, Shi Z and Ng HK. MIR-137 suppresses growth and invasion, is downregulated in oligodendroglial tumors and targets CSE1L. Brain Pathol 2013; 23: 426-439.

[16] Wang X, Ren Y, Ma S and Wang S. Circular RNA 0060745, a novel circRNA, promotes colorectal cancer cell proliferation and metastasis through miR-4736 sponging. Onco Targets Ther 2020; 13: 1941-1951.
[17] Li Y, Yuan S, Liu J, Wang Y, Zhang Y, Chen X and Si W. CSE1L silence inhibits the growth and metastasis in gastric cancer by repressing GPNMB via positively regulating transcription factor MITF. J Cell Physiol 2020; 235: 2071-2079.
[18] Zhang X, Zhang X, Mao T, Xu H, Cui J, Lin H and Wang L. CSE1L, as a novel prognostic marker, promotes pancreatic cancer proliferation by regulating the AKT/mTOR signaling pathway. J Cancer 2021; 12: 2797-2806.
[19] Wang YS, Peng C, Guo Y and Li Y. CSE1L promotes proliferation and migration in oral cancer through positively regulating MITF. Eur Rev Med Pharmacol Sci 2020; 24: 5429-5435.
[20] Lin HC, Li J, Cheng DD, Zhang X, Yu T, Zhao FY, Geng Q, Zhu MX, Kong HW, Li H and Yao M. Nuclear export protein CSE1L interacts with P65 and promotes NSCLC growth via NF-kappaB/MAPK pathway. Mol Ther Oncolytics 2021; 21: 23-36.
[21] Wang L, He Y, Liu W, Bai S, Xiao L, Zhang J, Dhanasekaran SM, Wang Z, Kalyana-Sundaram S, Balbin OA, Shukla S, Lu Y, Lin J, Reddy RM, Carrott PW Jr, Lynch WR, Chang AC, Chinnaiyan AM, Beer DG, Zhang J and Chen G. Noncoding RNA LINC00857 is predictive of poor patient survival and promotes tumor progression via cell cycle regulation in lung cancer. Oncotarget 2016; 7: 11487-11499.
[22] Su W, Feng S, Chen X, Yang X, Mao R, Guo C, Wang Z, Thomas DG, Lin J, Reddy RM, Orringer MB, Chang AC, Yang Z, Beer DG and Chen G. Silencing of long noncoding RNA MIR22HG triggers cell survival/death signaling via oncogenes YBX1, MET, and p21 in lung cancer. Cancer Res 2018; 78: 3207-3219.
[23] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.
[24] Chen G, Gharib TG, Thomas DG, Huang CC, Misek DE, Kuick RD, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM and Beer DG. Proteomic analysis of eIF-5A in lung adenocarcinomas. Proteomics 2003; 3: 496-504.
[25] Kim SH, Chen G, King AN, Jeon CK, Christensen PJ, Zhao L, Simpson RU, Thomas DG, Giordano TJ, Brenner DE, Hollis B, Beer DG and Ramnath N. Characterization of vitamin D receptor (VDR) in lung adenocarcinoma. Lung Cancer 2012; 77: 265-271.
[26] Albertus DL, Seder CW, Chen G, Wang X, Hartojo W, Lin L, Silvers A, Thomas DG, Giordano TJ, Chang AC, Orringer MB, Bigbee WL, Chinnaiyan AM and Beer DG. AZGP1 autoantibody predicts survival and histone deacetylase inhibitors increase expression in lung adenocarcinoma. J Thorac Oncol 2008; 3: 1236-1244.
[27] Su W, Wang L, Zhao H, Hu S, Zhou Y, Guo C, Wu B, Li L, Yang Z, Beer DG and Chen G. LINC00857 interacting with YBX1 to regulate apoptosis and autophagy via MET and phosphor-AMPKa signaling. Mol Ther Nucleic Acids 2020; 22: 1164-1175.
[28] Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma, Shedden K, Taylor JM, Enkemann SA, Tsao MS, Yeatman TJ, Gerald WL, Eschrich S, Jurisica I, Giordano TJ, Misek DE, Chang AC, Zhu CQ, Strumpf D, Hanash S, Shepherd FA, Ding K, Seymour L, Naoki K, Pennell N, Weir B, Verhaak R, Ladd-Acosta C, Golub T, Gruidl M, Sharma A, Szoke J, Zakowski M, Rusch V, Kris M, Viale A, Motoi N, Travis W, Conley B, Seshan VE, Meyerson M, Kuick R, Dobbin KK, Lively T, Jacobson JW and Beer DG. Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. Nat Med 2008; 14: 822-827.
[29] Su LJ, Chang CW, Wu YC, Chen KC, Lin CJ, Liang SC, Lin CH, Whang-Peng J, Hsu SL, Chen CH and Huang CY. Selection of DDX5 as a novel internal control for Q-RT-PCR from microarray data using a block bootstrap re-sampling scheme. BMC Genomics 2007; 8: 140.
[30] Tomida S, Takeuchi T, Shimada Y, Arima C, Matsuo K, Mitsudomi T, Yatabe Y and Takahashi T. Relapse-related molecular signature in lung adenocarcinomas identifies patients with dismal prognosis. J Clin Oncol 2009; 27: 2793-2799.
[31] Hou J, Aerts J, den Hamer B, van Ijcken W, den Bakker M, Riegman P, van der Leest C, van der Spek P, Foekens JA, Hoogsteden HC, Grosveld F and Philipsen S. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. PLoS One 2010; 5: e10312.
[32] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.
[33] Dhanasekaran SM, Balbin OA, Chen G, Nadal E, Kalyana-Sundaram S, Pan J, Veeneman B, Cao X, Malik R, Vats P, Wang R, Huang S, Zhong J, Jing X, Iyer M, Wu YM, Harms PW, Lin J, Reddy R, Brennan C, Palanisamy N, Chang AC, Truini A, Truini M, Robinson DR, Beer DG and Chinnaiyan AM. Transcriptome meta-analysis of lung cancer reveals recurrent aberrations in NRG1 and Hippo pathway genes. Nat Commun 2014; 5: 5893.

[34] Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014; 511: 543-550.
[35] Seo JS, Ju YS, Lee WC, Shin JY, Lee JK, Bleazard T, Lee J, Jung YJ, Kim JO, Shin JY, Yu SB, Kim J, Lee ER, Kang CH, Park IK, Rhee H, Lee SH, Kim JI, Kang JH and Kim YT. The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 2012; 22: 2109-2119.
[36] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ and Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010; 28: 511-515.
[37] Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.
[38] Saigi M, Alburquerque-Bejar JJ, Mc Leer-Florin A, Pereira C, Pros E, Romero OA, Baixeras N, Esteve-Codina A, Nadal E, Brambilla E and Sanchez-Cespedes M. MET-oncogenic and JAK2-inactivating alterations are independent factors that affect regulation of PD-L1 expression in lung cancer. Clin Cancer Res 2018; 24: 4579-4587.
[39] Ahn HK, Kim S, Kwon D, Koh J, Kim YA, Kim K, Chung DH and Jeon YK. MET receptor tyrosine kinase regulates the expression of co-stimulatory and co-inhibitory molecules in tumor cells and contributes to PD-L1-mediated suppression of immune cell function. Int J Mol Sci 2019; 20: 4287.
[40] Comoglio PM, Trusolino L and Boccaccio C. Known and novel roles of the MET oncogene in cancer: a coherent approach to targeted therapy. Nat Rev Cancer 2018; 18: 341-358.
[41] Guo R, Luo J, Chang J, Rekhtman N, Arcila M and Drilon A. MET-dependent solid tumours-molecular diagnosis and targeted therapy. Nat Rev Clin Oncol 2020; 17: 569-587.
[42] Recondo G, Che J, Janne PA and Awad MM. Targeting MET dysregulation in cancer. Cancer Discov 2020; 10: 922-934.
[43] Chin SY, Wu PR, Shih YH, Yeh CM, Lee WR, Shen SC, Yeh KT, Jiang MC and Tseng JT. High expression of cytoplasmic phosphorylated CSE1L in malignant melanoma but not in benign nevi: phosphorylated CSE1L for the discrimination between melanoma and benign nevi. Int J Clin Exp Pathol 2015; 8: 1393-1401.
[44] Jiang MC, Yeh CM, Tai CJ, Chen HC, Lin SH, Su TC, Shen SC, Lee WR, Liao CF, Li LT, Lee CH, Chen YC, Yeh KT and Chang CC. CSE1L modulates Ras-induced cancer cell invasion: correlation of K-Ras mutation and CSE1L expression in colorectal cancer progression. Am J Surg 2013; 206: 418-427.
[45] Lorenzato A, Martino C, Dani N, Oligschlager Y, Ferrero AM, Biglia N, Calogero R, Olivero M and Di Renzo MF. The cellular apoptosis susceptibility CAS/CSE1L gene protects ovarian cancer cells from death by suppressing RASSF1C. FASEB J 2012; 26: 2446-2456.
[46] Alnabulsi A, Agouni A, Mitra S, Garcia-Murillas I, Carpenter B, Bird S and Murray GI. Cellular apoptosis susceptibility (chromosome segregation 1-like, CSE1L) gene is a key regulator of apoptosis, migration and invasion in colorectal cancer. J Pathol 2012; 228: 471-481.
[47] Tai CJ, Shen SC, Lee WR, Liao CF, Deng WP, Chiou HY, Hsieh CI, Tung JN, Chen CS, Chiou JF, Li LT, Lin CY, Hsu CH and Jiang MC. Increased cellular apoptosis susceptibility (CSE1L/CAS) protein expression promotes protrusion extension and enhances migration of MCF-7 breast cancer cells. Exp Cell Res 2010; 316: 2969-2981.
[48] Pimiento JM, Neill KG, Henderson-Jackson E, Eschrich SA, Chen DT, Husain K, Shibata D, Coppola D and Malafa MP. Knockdown of CSE1L gene in colorectal cancer reduces tumorigenesis in vitro. Am J Pathol 2016; 186: 2761-2768.
[49] Tunccan T, Duzer S, Dilek G, Yuksel UM, Cetiner H, Kilic C, Ant A and Duran AB. The role of CSE1L expression in cervical lymph node metastasis of larynx tumors. Braz J Otorhinolaryngol 2019; 87: 42-46.
[50] Chang CC, Tai CJ, Su TC, Shen KH, Lin SH, Yeh CM, Yeh KT, Lin YM and Jiang MC. The prognostic significance of nuclear CSE1L in urinary bladder urothelial carcinomas. Ann Diagn Pathol 2012; 16: 362-368.
[51] Cheng DD, Lin HC, Li SJ, Yao M, Yang QC and Fan CY. CSE1L interaction with MSH6 promotes osteosarcoma progression and predicts poor patient survival. Sci Rep 2017; 7: 46238.
[52] Shiraki K, Fujikawa K, Sugimoto K, Ito T, Yamanaka T, Suzuki M, Yoneda K, Sugimoto K, Takase K and Nakano T. Cellular apoptosis susceptibility protein and proliferation in human hepatocellular carcinoma. Int J Mol Med 2006; 18: 77-81.
[53] Yuksel UM, Dilek G, Dogan L, Gulcelik MA and Berberoglu U. The relationship between CSE1L expression and axillary lymph node metastasis in breast cancer. Tumori 2015; 101: 194-198.
[54] Liao CF, Lin SH, Chen HC, Tai CJ, Chang CC, Li LT, Yeh CM, Yeh KT, Chen YC, Hsu TH, Shen SC, Lee WR, Chiou JF, Luo SF and Jiang MC. CSE1L, a novel microvesicle membrane protein, mediates Ras-triggered microvesicle generation and metastasis of tumor cells. Mol Med 2012; 18: 1269-1280.

[55] Tung MC, Tsai CS, Tung JN, Tsao TY, Chen HC, Yeh KT, Liao CF and Jiang MC. Higher prevalence of secretory CSE1L/CAS in sera of patients with metastatic cancer. Cancer Epidemiol Biomarkers Prev 2009; 18: 1570-1577.
[56] Ma S, Yang D, Liu Y, Wang Y, Lin T, Li Y, Yang S, Zhang W and Zhang R. LncRNA BANCR promotes tumorigenesis and enhances adriamycin resistance in colorectal cancer. Aging (Albany NY) 2018; 10: 2062-2078.
[57] Liu C, Wei J, Xu K, Sun X, Zhang H and Xiong C. CSE1L participates in regulating cell mitosis in human seminoma. Cell Prolif 2019; 52: e12549.
[58] Lamberti G, Sisi M, Andrini E, Palladini A, Giunchi F, Lollini PL, Ardizzoni A and Gelsomino F. The mechanisms of PD-L1 regulation in Non-Small-Cell Lung Cancer (NSCLC): which are the involved players? Cancers (Basel) 2020; 12: 3129.
[59] Su C, Wang H, Liu Y, Guo Q, Zhang L, Li J, Zhou W, Yan Y, Zhou X and Zhang J. Adverse effects of anti-PD-1/PD-L1 therapy in non-small cell lung cancer. Front Oncol 2020; 10: 554313.

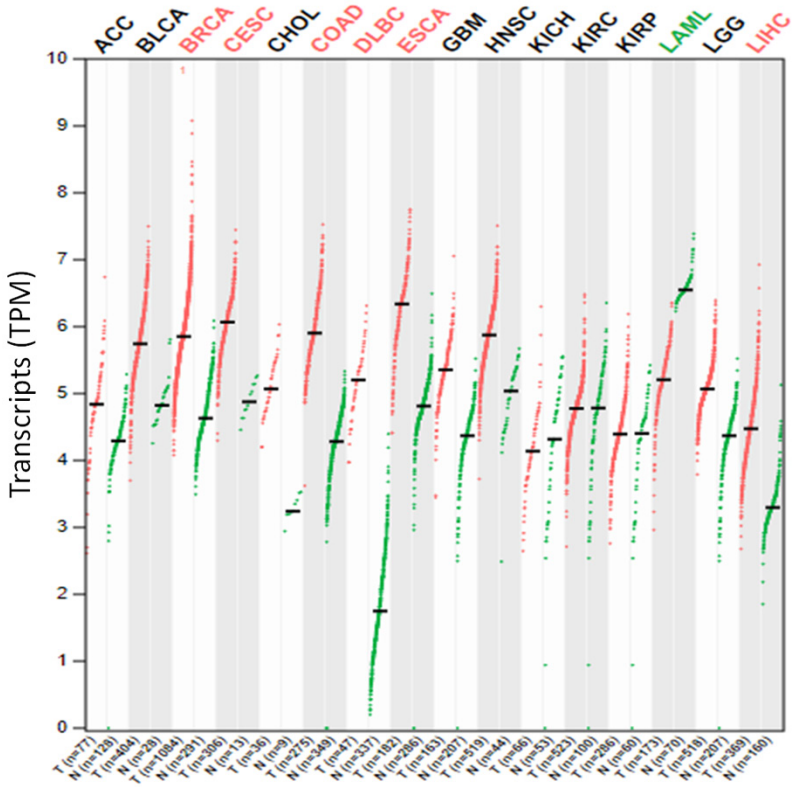

Figure S1. *CSE1L* mRNA expression in different cancer types and its matched normal tissues. This figure is from GEPIA website of TCGA RNA-seq data. Abbreviations: ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangio carcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma.

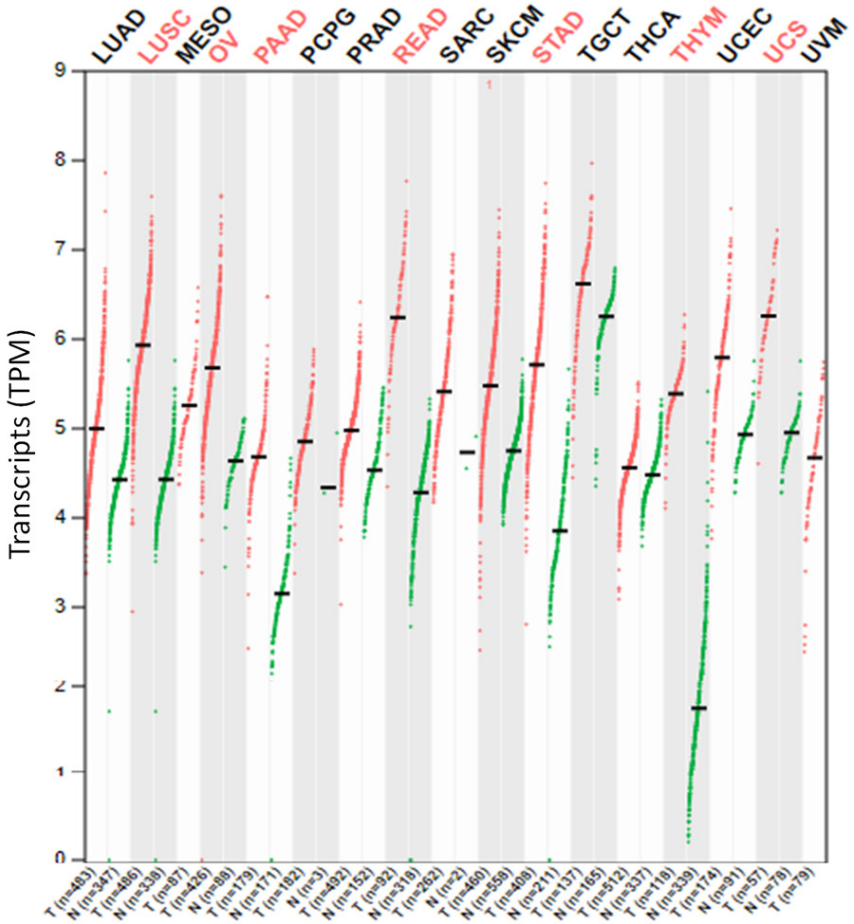

Figure S2. *CSE1L* mRNA expression in different cancer types and its matched normal tissues (continues to Figure S1). This figure is from GEPIA website of TCGA RNA-seq data. Abbreviations: LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyzoid carcinoma; THYM, Thyihoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, Uveal Melanoma.

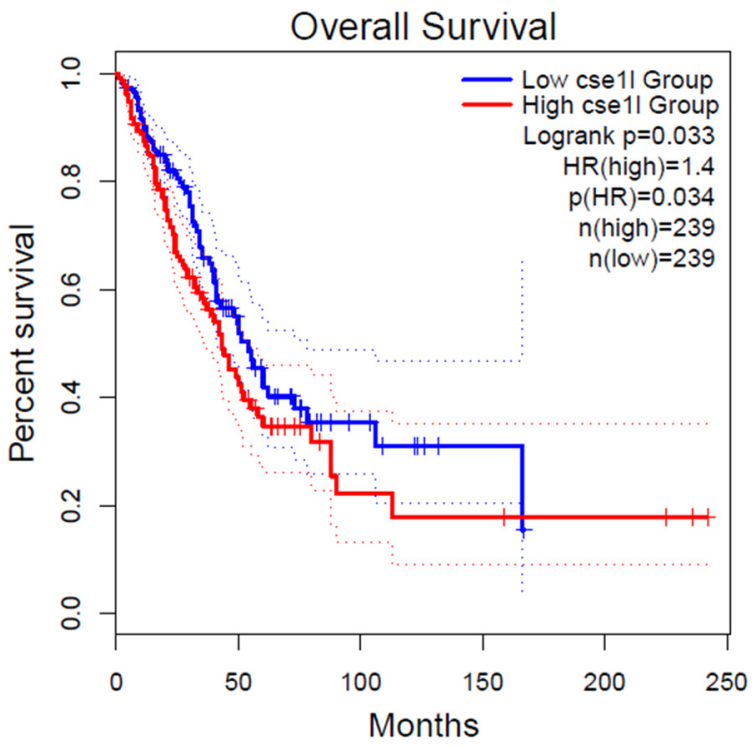

Figure S3. The higher *CSE1L* mRNA level is correlated with unfavorable survival in patients with AD. The Kaplan-Meier survival curve is from GEPIA website of TCGA lung adenocarcinoma RNA-seq data. Log-rank test, *P*=0.033.

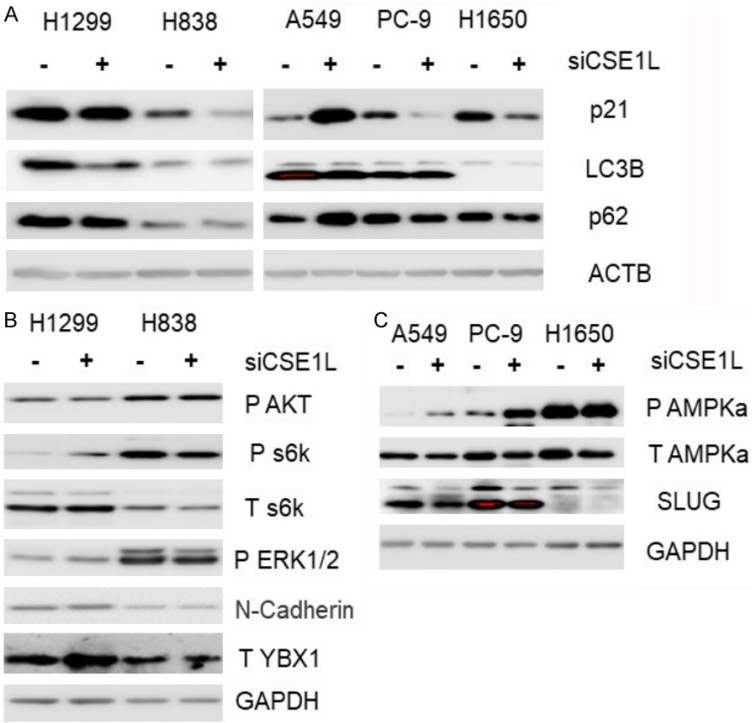

Figure S4. A-C. Western blot showing proteins changing up CSE1L siRNA treatment at 72 h in lung cancer cell lines. Note: red lines in bands of LC3B and SLUG were caused by ChemiDoc MP Imaging System indicting more saturation. p21 was decreased in H838, PC-9 and H1650, while increased in A549 and not changed in H1299. Autophagy related proteins such as LC3B, p62 and AMPKa were not changed. Other proteins such as p-AKT, s6k, p-ERK1/2, N-cadherin, YBX1 and SLUG were not changed upon CSE1L knockdown.